Evidence of the association of *BIN1* and *PICALM* with the AD risk in contrasting European populations

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Abstract

Recent genome-wide association studies have identified five loci (*BIN1*, *CLU*, *CR1*, *EXOC3L2* and *PICALM*) as genetic determinants of Alzheimer's disease (AD). We attempted to confirm the association between these genes and the AD risk in three contrasting European populations (from Finland, Italy and Spain). Since *CLU* and *CR1* had already been analyzed in these populations, we restricted our investigation to *BIN1*, *EXO2CL3* and *PICALM*. In a total of 2,816 AD cases and 2,706 controls, we unambiguously replicated the association of rs744373 (for BIN1) and rs541458 (for PICALM) polymorphisms with the AD risk (OR=1.26, 95% CI [1.15-1.38], $p=2.9x10^{-7}$, and OR=0.80, 95% CI [0.74-0.88], $p=4.6x10^{-7}$, respectively). In a meta-analysis, rs597668 (EXOC3L2) was also associated with the AD risk, albeit to a lesser extent (OR=1.19, 95% CI [1.06-1.32], $p=2.0x10^{-3}$). However, this signal did not appear to be independent of *APOE*.

In conclusion, we confirmed that *BIN1* and *PICALM* are genetic determinants of AD, whereas the potential involvement of EXOC3L2 requires further investigation.

Introduction

Although Alzheimer's disease (AD) is the most common cause of dementia in the elderly, its aetiology is still not fully understood. The characterization of causative factors is thus important for better defining the pathophysiological processes involved. In this context, the identification of genes involved in monogenic forms of AD has significantly contributed to our knowledge of the disease mechanisms (Bettens, 2010). In contrast, the characterization of genetic factors involved in the common forms of AD (i.e. lacking classical Mendelian inheritance) has encountered significant difficulties; the apolipoprotein E (APOE) gene is the only globally valid genetic determinant of AD to have been unambiguously identified in 15 years of intensive research (Lambert, 2007).

However, as with other multifactorial diseases, this systematic inability to detect new genetic determinants has prompted more comprehensive investigations using genome-wide association studies (GWASs). We and others performed three large GWASs in this field and reported that the *CLU* (clusterin), *PICALM* (phosphatidylinositol binding clathrin assembly protein), *CR1* (complement component [3b/4b] receptor 1), *BIN1* (bridging integrator 1) and *EXOC3L2* (exocyst complex component 3-like 2) loci were associated with the AD risk (Harold, 2009; Lambert, 2009; Seshadri, 2010).

To help to clarify the relevance of these genes as genetic determinants of AD, we analyzed their associations in contrasting European populations from Finland (n=1,123), Italy (n=2,811) and Spain (n=1,588). Since CLU and CR1 have been already studied in these populations (Lambert, 2009), we only tested single-nucleotide polymorphisms (SNPs) within *PICALM*, *BIN1* and *EXOC3L2*.

Materials and Methods

All clinical diagnoses of probable AD were established according to the DSM-III-R and NINCDS-ADRDA criteria. Controls were defined as subjects not meeting the DMS-III-R dementia criteria and with intact cognitive functions (MMS>25). Written, informed consent was obtained from study participants or, for those with substantial cognitive impairment, from a caregiver, legal guardian, or other proxy. The study protocols for all populations were reviewed and approved by the appropriate independent ethics committees in each country. Information on age and gender in the cases and controls included in the study are shown in Table 1. Samples with missing age or gender data were excluded, yielding a total of 2,816 AD cases and 2,706 controls.

Genotyping for the SNPs (rs744373 in *BIN1*, rs597668 in *EXOC3L2* and rs541458 in *PICALM*) was performed with a Taqman system (Applied Biosystems). The primer and probe sequences are available on request. In order to avoid bias, cases and controls were randomly mixed when genotyping and the laboratory personnel were blinded to case/control

status. The genotyping success rate was at least 95% and no departure from Hardy-Weinberg equilibrium was observed for the markers (Table 2).

We undertook logistic regression analyses in each country (Finland, Italy and Spain) using an additive genetic model which took account of age, gender, disease status and (when necessary) centre. All analyses were performed with SAS software (release 9.1, SAS Institute, Cary, NC, USA). We then used inverse-variance weighting (also known as fixed-effects meta-analysis) with adjustments for age and gender for the overall effect assessment, using Review Manager software (release 5.0). Interactions between *BIN1, EXOC3L2, PICALM* and *APOE* ε4 polymorphisms were tested in logistic regression models adjusted for age, gender and (when necessary) centre. We again used inverse-variance weighting, with adjustments for age and gender for assessment of the overall interaction. Linkage disequilibrium was assessed using Haploview software.

Results

In each data set, we evaluated the association of AD with the rs744373, rs597668 and rs541458 SNPs within the *BIN1*, *EXOC3L2* and *PICALM* loci, respectively. Even though the detected associations were not always statistically significant in all data sets, they were comparable in direction in the three different European populations. When the data sets were examined in a meta-analysis, we found strong evidence of associations for *BIN1* (OR=1.26, $p=2.9x10^{-7}$), *PICALM* (OR=0.80, $p=4.7x10^{-7}$) and, to a lesser extent, *EXOC3L2* (OR=1.19, $p=2.0x10^{-3}$) (Table 3).

We also searched for significant interactions between these three loci and APOE but failed to identify any in either the independent data sets or in the meta-analysis. We nevertheless re-evaluated the association of the *BIN1*, *PICALM* and *EXOC3L2* SNPs with the AD risk by adjusting for age, gender and the presence of at least one APOE ε4 allele. Whereas the *BIN1* and *PICALM* associations were not modified (data not shown), we found no evidence of an association of *EXOC3L2* with the AD risk after adjustment of the data sets taken individually (Finland, OR=0.91, $p=4.2x10^{-1}$; Italy, OR=0.99, $p=8.9x10^{-1}$; Spain, OR=1.06, $p=6.2x10^{-1}$) or in the meta-analysis (OR=0.98, $p=7.8x10^{-1}$).

Discussion

Over recent months, our picture of the genetics of AD has changed greatly and suggests that most of the genuine genetic determinants of this disease will differ from those suspected before the advent of the GWASs (Laumet, 2010; Sleegers, 2010). By looking at contrasting European populations of AD cases and controls in which the associations of *CLU* and *CR1* with AD had already replicated (Lambert, 2009), we confirmed the association of *PICALM* and *BIN1* with the AD risk. The ORs are comparable in direction and magnitude with those

originally reported. The association of *PICALM* with the AD risk has been already replicated in several large data sets (Carrasquillo, 2010; Corneveaux, 2010; Jun, in press; Kamboh, in press; Seshadri, 2010). Using the ORs reported in the AlzGene database (http://www.alzgene.org) (Bertram, 2007) and by including our new data, a meta-analysis unambiguously showed that this gene is a genuine risk factor for AD (OR=0.87, 95%CI [0.84-0.90], $p=5.5x10^{-18}$, p for heterogeneity=0.27).

To the best of our knowledge, our study is the first to have replicated the association of the *BIN1* and *EXOC3L2* loci with the AD risk. The meta-analysis of our data and those gathered by Seshadri et al. strongly supported the involvement of the *BIN1* gene in AD (OR=1.16, 95%CI [1.12-1.21, $p=1.6x10^{-15}$, p for heterogeneity=0.14). However, in contrast to Seshadri et al.'s report, we were unable to show that the *EXOC3L2* signal was independent of the *APOE* locus. Surprisingly, and even though this gene locus is close to *APOE*, we did not detect linkage disequilibrium between the *APOE* ε4 allele and the rs597668 SNP (D'=0.36 and r^2=0.075 at most, in the Finland data set). This locus thus deserves more attention, in order to confirm or refute its association with the AD risk.

In conclusion, we unambiguously replicated the association of the *PICALM* and *BIN1* loci (both of which code for proteins involved in endocytosis and clathrin-mediated synaptic vessel formation (Harel, 2008; Wigge, 1997)) with the AD risk in contrasting European populations. In order to determine the exact implication of *BIN1* and *PICALM* in AD, it is now essential to develop major, systematic, ambitious efforts in sequencing and genotyping (even for rare variants), together with replication in large, independent populations and functional analyses of intermediate phenotypes.

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Table 1: characteristics of the different case-control studies according to countries

	Finland (1 centre)		Italy (10 centres)		Spain (3 centres)	
	AD cases	Controls	AD cases	controls	AD cases	controls
n	589	541	1 520	1 291	755	833
Mean age	71.3 ± 7.4	69.0 ± 6.4	76.6 ± 8.7	72.3 ± 8.9	75.3 ± 9.3	76.9 ± 10.9
Mean age at onset	71.3 ± 7.4	-	73.8 ± 8.8	-	72.5 ± 9.4	-
% male	32	42	32	45	43	38

Table 2: Genotype distribution of rs744373, rs597668 and rs541458 in AD cases and controls

rs744373	**Genotype distribution** freq. (*n*)					
	AA		**AG**		**GG**	
Finland						
Controls *(529)*	0.603	*(319)*	0.337	*(178)*	0.060	*(32)*
Cases *(563)*	0.572	*(322)*	0.368	*(207)*	0.060	*(34)*
Italy						
Controls *(1265)*	0.535	*(677)*	0.399	*(504)*	0.066	*(84)*
Cases *(1460)*	0.489	*(714)*	0.431	*(629)*	0.080	*(117)*
Spain						
Controls *(829)*	0.551	*(457)*	0.395	*(327)*	0.054	*(45)*
Cases *(726)*	0.463	*(336)*	0.423	*(307)*	0.114	*(83)*
rs597668	**Genotype distribution** freq. (*n*)					
	TT		**TC**		**CC**	
Finland						
Controls *(529)*	0.571	*(302)*	0.348	*(184)*	0.081	*(43)*
Cases *(562)*	0.471	*(265)*	0.429	*(241)*	0.100	*(56)*
Italy						
Controls *(1268)*	0.773	*(980)*	0.210	*(266)*	0.017	*(22)*
Cases *(1457)*	0.765	*(1115)*	0.219	*(336)*	0.140	*(90)*
Spain						
Controls *(832)*	0.804	*(669)*	0.180	*(150)*	0.016	*(13)*
Cases *(727)*	0.769	*(559)*	0.212	*(54)*	0.019	*(14)*
rs541458	**Genotype distribution** freq. (*n*)					
	TT		**TG**		**GG**	
Finland						
Controls *(521)*	0.403	*(210)*	0.430	*(225)*	0.167	*(86)*
Cases *(561)*	0.438	*(246)*	0.437	*(245)*	0.125	*(70)*
Italy						
Controls *(1257)*	0.439	*(552)*	0.446	*(561)*	0.115	*(144)*
Cases *(1460)*	0.515	*(752)*	0.406	*(592)*	0.080	*(116)*
Spain						
Controls *(819)*	0.492	*(403)*	0.419	*(343)*	0.089	*(73)*
Cases *(723)*	0.546	*(395)*	0.391	*(283)*	0.062	*(45)*

Table 3: Association of rs744373, rs597668 and rs541458 with the AD risk in Finnish, Italian and Spanish case-control studies. (HW, hardy-Weinberg; MAF, minor allele Frequency)

rs744373	N		MAF		HW	Association Test		*APOE* interaction
(BIN1)	Cases	Controls	Cases	Controls		OR (95% CI)	*P* value	*P value*
Finland	563	529	0.24	0.23	$2.9x10^{-1}$	1.12 (0.92-1.37)	$2.6x10^{-1}$	$8.5x10^{-1}$
Italy	1 460	1 265	0.30	0.27	$4.5x10^{-1}$	1.22 (1.07-1.38)	$2.0x10^{-3}$	$6.6x10^{-1}$
Spain	726	829	0.33	0.25	$1.7x10^{-1}$	1.43 (1.22-1.68)	$1.4x10^{-6}$	$7.3x10^{-1}$
Meta-analysis[1]						**1.26 (1.15-1.38)**	**$2.9x10^{-7}$**	$6.8x10^{-1}$
rs597668	N		MAF			Association Test		*APOE* interaction
(EXO3CL2)	Cases	Controls	Cases	Controls		OR (95% CI)	*P* value	*P value*
Finland	562	529	0.31	0.26	$5.0x10^{-2}$	1.38 (1.14-1.66)	$9.0x10^{-4}$	$5.9x10^{-1}$
Italy	1 457	1 268	0.13	0.12	$4.2x10^{-1}$	1.05 (0.89-1.24)	$5.7x10^{-1}$	$6.3x10^{-2}$
Spain	727	832	0.13	0.11	$1.8x10^{-1}$	1.19 (0.96-1.48)	$1.1x10^{-1}$	$7.9x10^{-1}$
Meta-analysis[1]						**1.19 (1.06-1.32)**	**$2.0x10^{-3}$**	$1.1x10^{-1}$
rs541458	N		MAF			Association Test		*APOE* interaction
(PICALM)	Cases	Controls	Cases	Controls		OR (95% CI)	*P* value	*P value*
Finland	561	521	0.34	0.38	$6.0x10^{-2}$	0.85 (0.71-1.01)	$6.8x10^{-2}$	$9.0x10^{-1}$
Italy	1 460	1 257	0.30	0.26	$9.4x10^{-1}$	0.78 (0.69-0.88)	$5.1x10^{-5}$	$6.6x10^{-1}$
Spain	723	819	0.26	0.30	$1.0x10^{-1}$	0.81 (0.69-0.95)	$1.1x10^{-2}$	$7.1x10^{-1}$
Meta-analysis[1]						**0.80 (0.74-0.88)**	**$4.6x10^{-7}$**	$9.7x10^{-1}$

[1] inverse-variance weighting with adjustments for age, gender and centre when necessary
A fixed effect model was used (p-value for heterogeinity not significant whatever the SNP analysed, p=0.12 for BIN1, p=0,11 for EXO2CL3 and p=0.68 for PICALM)